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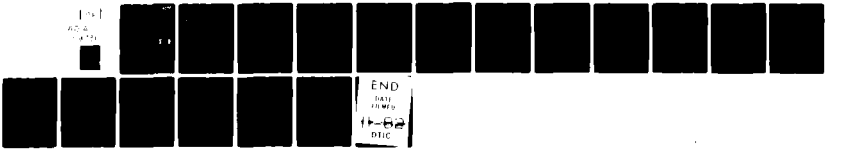

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Simian virus 40 DNA was irradiated by a high intensity ArF excimer laser. Analysis by gel electrophoresis, neutral sucrose gradient analysis and alkaline sucrose gradient analysis showed that the laser irradiation leads to breaking of the sugar phosphate backbone in a sequential manner at random sites. The causitive process is either attack by laser generated free radicals or two photon direct scission.

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Laser Irradiation of SV40 DNA

by

Marian Johnson-Thompson, Joshua B. Halpern

William M. Jackson and Jay George

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September 17, 1982

LASER IRRADIATION OF SV40 DNA

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Modern photochemical studies of DNA have concentrated on the absorption of the bases at about 260 nm and subsequent dimerization and photorepair mechanisms (1-5). Lethokov has pointed out that there may be important differences when high intensity UV lasers are used to irradiate the DNA (6). Preliminary results from his group are encouraging in this respect, showing interesting changes in the absorption spectrum of bases irradiated by such lasers which can be attributed to multiphoton absorption in the molecules (7).

We have irradiated Simian Virus 40 (SV40) DNA with an ArF laser. The results of conformational analysis showed an initial conversion of the double stranded superhelical native form to a form with a single circular and a single linear strand indicating cleavage in one strand. Further irradiation leads to sequential fragmentation of both strands. This type of photoalteration is more typical of the effect of vacuum ultraviolet light (8) and X-Rays (9) on DNA than that of near UV photons. The 193 nm photons are orders of magnitude less energetic than the X-rays, so that even if two or more photons are needed, the amount of energy injected into the DNA molecule is much smaller. As a consequence the photoeffect should be easier to control and have more specificity. VUV photons on the other hand are hard to generate and impossible to propagate through the atmosphere, and almost all solvents, requiring that one work in vacuum. Multiphoton excitation schemes using lasers would access the same energy levels but be easier to work with.

Most previous photochemical studies of DNA have been performed at 260 nm. However, the extinction coefficients for the band at 200 nm is much greater than that for the 260 nm band. Tanaka and Nagakura have measured the absorption spectra of adenine and thymine in the region of 160 to 280 nm (10). Nakanishi measured the absorption of uracil (11) and absorption of other bases has been measured by Clark and Tinoco (12). In all cases the absorption coefficient at the wavelength of the ArF laser is substantial and can be as much as t n times greater than the absorption coefficient at 260 nm.

Jung, et al. (8) measured the effects of VUV radiation on DNA

from phage ØX174. Above 124 nm the UV radiation damages the bases, while below this limit strand breaks deriving from ionization are the primary damage mechanism. Thus the kinds of strand breaks that we observe would require the absorption of two photons, at a site, or concentration of the energy absorbed at two random sites. Possible mechanisms for this will be discussed below.

SV40 DNA, is obtained from a small DNA tumor virus of the Papovavirus group which is one of the most thoroughly characterized of the DNA containing viruses. The nucleic acid has a unique conformation, existing as a covalently closed superhelical duplex (13) with a molecular weight of about 3.5×10^6 daltons (14,15). Following extraction of SV40 DNA from the virus, the DNA is obtained as Form I which is a covalently closed superhelical duplex. If the Form I DNA is damaged so that there is a nonspecific nick in one strand the conformation will relax to form a circular duplex.

Irradiation of Simian Virus 40 DNA with an ArF laser has yielded intriguing results. Two sets of studies have been done. In the first the changes of the absorption spectra of DNA in water was measured as a function of irradiation dose. In the second, conformational changes in the structure of SV40 DNA were measured as a function of irradiation.

Figure 1 shows the absorption spectrum of SV40 DNA in water measured as a function of irradiation time. The concentration of the DNA was 2×10^{-9} M and the laser was operated at 10 Hz with a fluence per shot of 7.5 mj/cm^2 and intensity of 3.7×10^5 Watts/cm^2. Absorption spectra were measured in a Beckman Acta double beam spectrometer. The sharp cutoff and modulation below 195 nm is attributable to a combination of absorption in the quartz cells and absorption of the Schumann-Runge bands of oxygen molecules. The reference arm of the instrument held a matched quartz absorption cell with water in it. The absorbance of the unirradiated sample is 0.73 through a 1 cm path length, with a molecular DNA density of 10^{12} molecules/cm^3. This corresponds to an absorption coefficient of 1.7×10^{-12} cm^2, an absolutely huge number attributable to the polymeric nature of the DNA. However, when one considers the number of bases in each DNA molecule, the absorption per base is seen

to be on the order of 10^{-17} cm^2, which is quite reasonable for a molecular absorption.

The ratio of the absorption coefficient at 200 nm to that at 260 nm is consistant with that seen in other DNAs. Figure 1 shows that a strong feature at about 230 nm appears when the sample is irradiated. This can be attributed to the formation of dimers in the bases.

Tritiated SV40 DNA in its native form was diluted in 2 ml of TES buffer at a specific activity of 2×10^5 dpm/g. The DNA solution was exposed to a 10 Hz ArF laser with an energy fluence of 50 mj/cm^2. The laser pulse length was 20 ns, meaning that the intensity of the laser radiation was 2.5×10^6 $Watts/cm^2$. Following irradiation, conformational analysis showed a specific photolytic conversion of the native, Form I DNA to Form II, corresponding to a single break in the backbone of one of the strands.

Simian Virus 40 DNA naturally exists in two forms. Form I, is the native form and is a double stranded molecule with a superhelical conformation (14,16). Form II is obtained from Form I by the introduction of a break in one of the strands, and no longer has a superhelical structure. A third form, Form III, can be produced in the laboratory by nicking both strands in the same location. Beyond this intense laser radiation has the ability to reduce the molecule to many short fragments, via multiple photodissociative processes which can occur either simultaneously or sequentially.

Just as important as the efficent conversion of Form I to Form II is the seeming resistance of the Form II DNA to conversion to Form III, in which both strands are broken at the same place. In the experiments that were done no Form III DNA was observed.

Figure 2 shows the results of neutral sucrose velocity sedimentation of control and laser irradiated SV40 DNA. In the control, 84% of the material sedimented at 21S, corresponding to Form I, while 16% sedimented at 16S, corresponding to Form II. This small amount is the result of

breaks in one strand of the DNA which occur during normal handling. No Form III DNA, which would sediment at 14S, is seen. The six minute irradiated sample shows a greater than fourfold decrease in the Form I SV40 DNA, while the amount of Form II DNA increases more than threefold. Further irradiation leads to an almost total disappearance of the Form I DNA.

The DNA samples were also analyzed on alkaline sucrose gradients which destroy the hydrogen bonds, and thus will separate the two strands of any DNA molecule if it has one or more breaks. This type of treatment can identify multiple nicks in both strands, as well as the different types of SV40 DNA. Figure 3 show the results of this analysis of the control and six and twelve minute irradiated samples. Again, the Form I DNA, sedimenting at 53S in this case, is seen to disappear rapidly. Form II DNA will sediment as a single circular strand at 18S and a linear strand at 16S. The hump that forms at the higher fraction numbers can be ascribed to smaller fragments resulting from multiple nicks in one strand of the DNA molecule. The formation of such fragments is not particularly surprising given the intensity of the laser.

Figure 3 shows that in the six minute sample there are more linear strands than circular ones. If only Form II DNA were sedimenting at 16S then this peak would be at the same height as the 18S peak. We interpret the difference in peak height to represent DNA molecules with a break in each strand, but with the breaks not at complimentry base pair sites. This must be so since no Form III DNA was seen in Figure 2. Such a molecule would maintain the circular shape of Form II, but destruction of the hydrogen bonds would result in sedimentation of two strands at 16S.

With increasing irradiation time it is seen that the difference between the 16S and 18S peak heights increases. This is clearly the result of the sequential photolysis of Form II DNA resulting in a single break in the other strand. Such products would sediment as Form II DNA in the neutral sucrose gradient analysis because the shape of the molecule would be about the same as Form II. We will refer to this type of DNA as Form II'. On the other hand there is no reason to assume that the sequential cleavage process must occur on the unbroken circular strand. If it occurs on the broken strand then alkaline gradient velocity sedimentation

will result in the production of two fragments, each of whose lengths will be less than that of a single strand, and which will sediment at lower Svedberg numbers. This is what we observe. Whether the site of the nth cleavage is completely random with respect to that of the (n+1)st is not known. For example, if absorption is significantly stronger at one of the bases, then one would expect the cleavages to form at those sites.

It can be seen that a lower dose than six minutes will result in a substantial amount of Form II DNA being created without the production of many small fragments, or of Form II'. It might be possible to almost totally convert the Form I DNA to Form II without the production of fragments, by controlling the intensity or the wavelength of the light, or both. This is a question we hope to explore.

The net effect of the laser radiation is to convert Form I DNA into Form II DNA, which may in turn be converted into smaller fragments by far UV photons. On the other hand, we have not ruled out the possibility of a photochemically initiated free radical reaction which could result in the breaking of the DNA molecular backbone. Breaks in the DNA chain can be produced by the attack of hydroxy radicals on the DNA for example (18). The intensity of the laser is such that a significant amount of free radicals could be created, even though the probability per photon for this to happen were very small. Possible sources are photolysis of dissolved oxygen molecules, photolysis of the water itself, or the photolysis of the buffer solution.

We do not however believe that this mechanism is operating. Creation of oxygen atoms is unlikely because the cavity of the excimer laser was not purged with nitrogen, so that emission in the region of the weak molecular oxygen absorption was supressed. The absorption of water above 185 nm is negligible, so that the photolysis would have to be a two photon process. Such processes have absorption cross-sections smaller than 10^{-48} cm^4/sec, which would imply that less than one in 10^8 molecules is dissociated per pulse in the experiments where the conformation of the DNA was determined. The density would be a factor of 25 lower in the absorption experiments. This does assume that the cell is optically thin.

When one compares the number of strand breaks as measured by conformational analysis against the number of photons absorbed as determined by measuring the absorption coefficient at 193nm the question yield for strand breaking is seen to about 10^{-5} per photon absorbed.

Many of the photons absorbed in the bases cause the formation of dimers as shown by the rise of the absorption coefficient at 230nm in Figure 1. Much of the absorbed energy is undoubtedly randomized within the vibrational manifold of the bases and the DNA chain. The low quantum yield and the energy of two 193nm laser photons are consistent with the hypothesis that the chain breakage is caused by simultaneous absorption of two photons in the sugar phosphate backbone.

In conclusion, large changes in the absorption spectra of control and irradiated SV40 DNA have been observed when an intense ArF laser was used to illuminate a dilute solution of Simian Virus 40 DNA. This was followed by absorption of further photons causing sequential fragmentation of the molecule.

FIGURE CAPTIONS

Figure 1. Absorption spectrum of SV40 DNA in water before and after irradiation.

Figure 2. Neutral sucrose velocity sedimentation of laser irradiated DNA I. Following irradiation, 25µl of each sample was layered separately onto 5-30% neutral sucrose (in 0.01 M Tris, 0.01 M EDTA, 0.05 M NaCl) gradients and centrifuged at 55,000 rpm for 2.15 hr. in a Beckman 60ti rotor at 5°C. Fractions (3drop) were collected directly into scintillation vials and counted. Fig. 2A, 2B and 2C represent control, 6 min laser irradiated and 12 min. laser irradiated samples, respectively. Sedimentation is from right to left.

Figure 3. Alkaline sucrose velocity sedimentation of laser irradiated DNA I. Following irradiation, 400µl of each sample was layered separately onto 10-30% alkalin sucrose (in 0.7 M NaCl, 0.3M NaOH, 0.01 M Tris, 0.0025 M EDTA, 0.015% Sarkosyl) gradients and centrifuged at 40,000 rpm for 17 hr. at 10°C in a Beckman SW41 rotor. Fractions (0.25 ml) were collected directly into scintillation vials. 100µl of glacial acetic acid was added to each and fractions were counted. Fig. 3A, 3B and 3C represent control, 6 min laser irradiated and 12 min laser irradiated, respectively. Sedimentation is from right to left.

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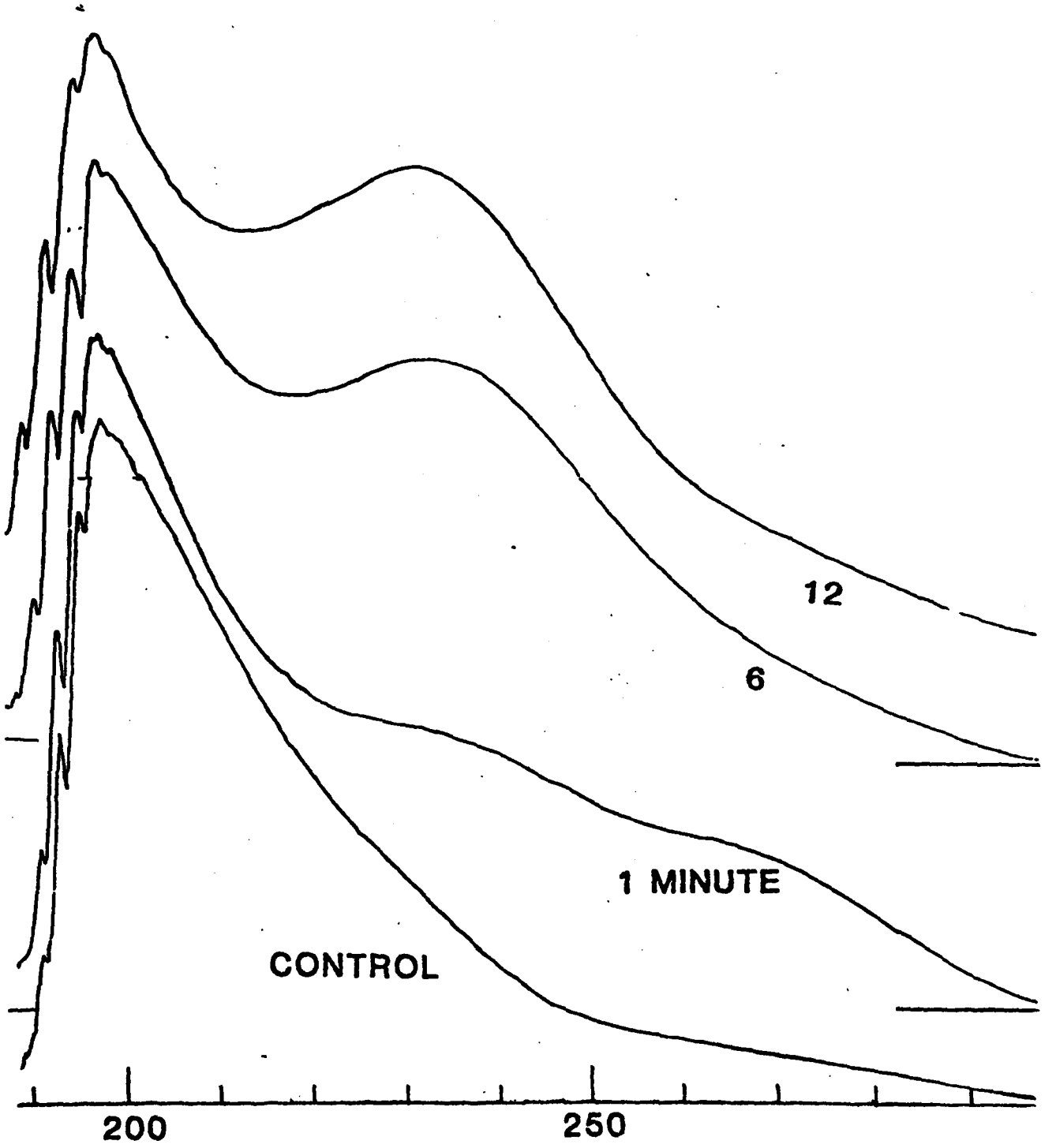
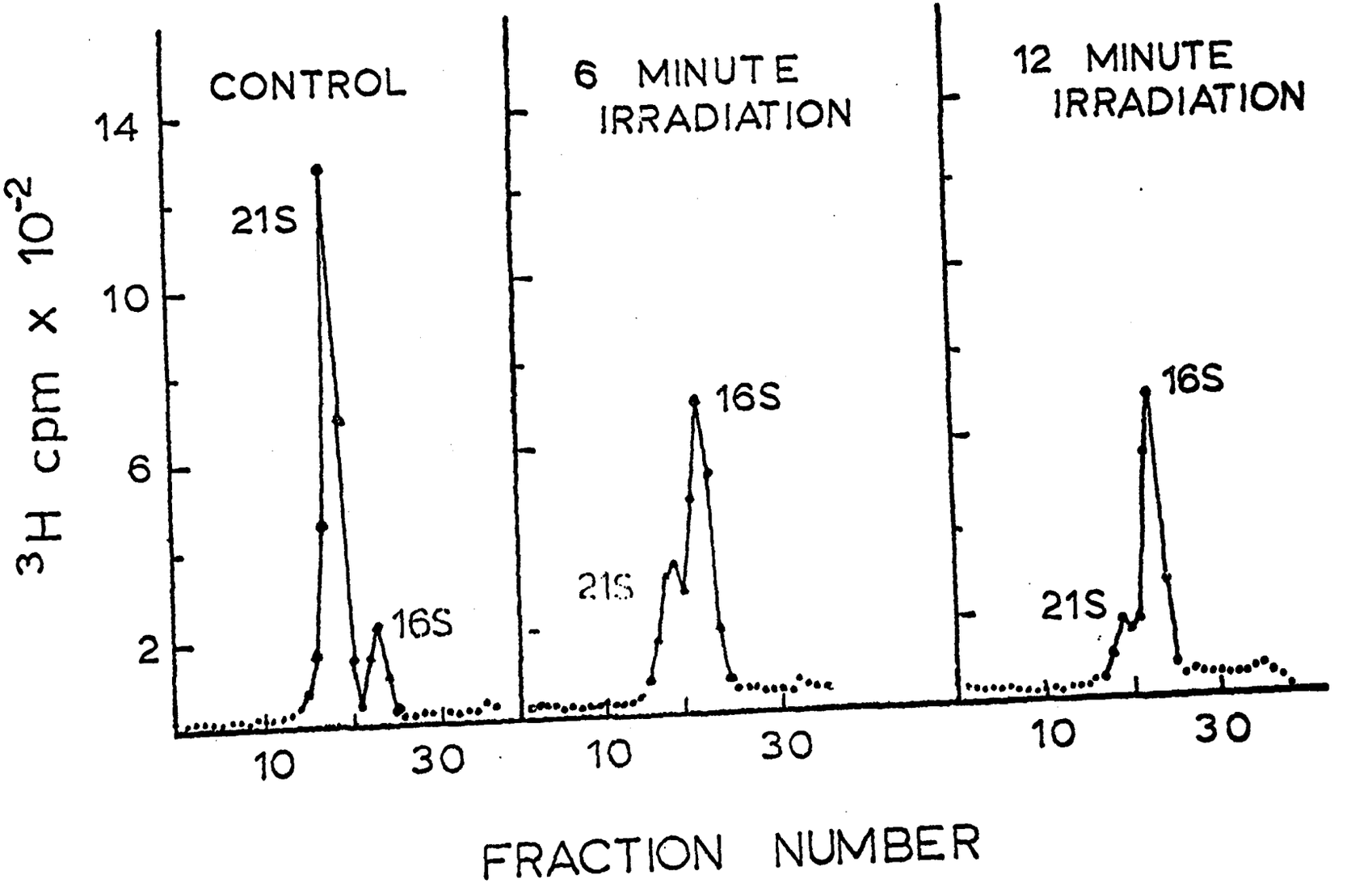
TABLE I

	Counts Per Min.			Percent	
	Comp. I	Comp. II	Total	Comp. I	Comp. II
Control	2922	576	3498	84%	16%
Laser 6'	1160	2235	3395	66%	34%
Laser 12'	516	2045	2561	20%	80%

ABSORPTION OF DNA IN WATER




CONTROL
6 MINUTE IRRADIATION
12 MINUTE IRRADIATION
^{3}H cpm x 10^{-2}
14
10
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21S
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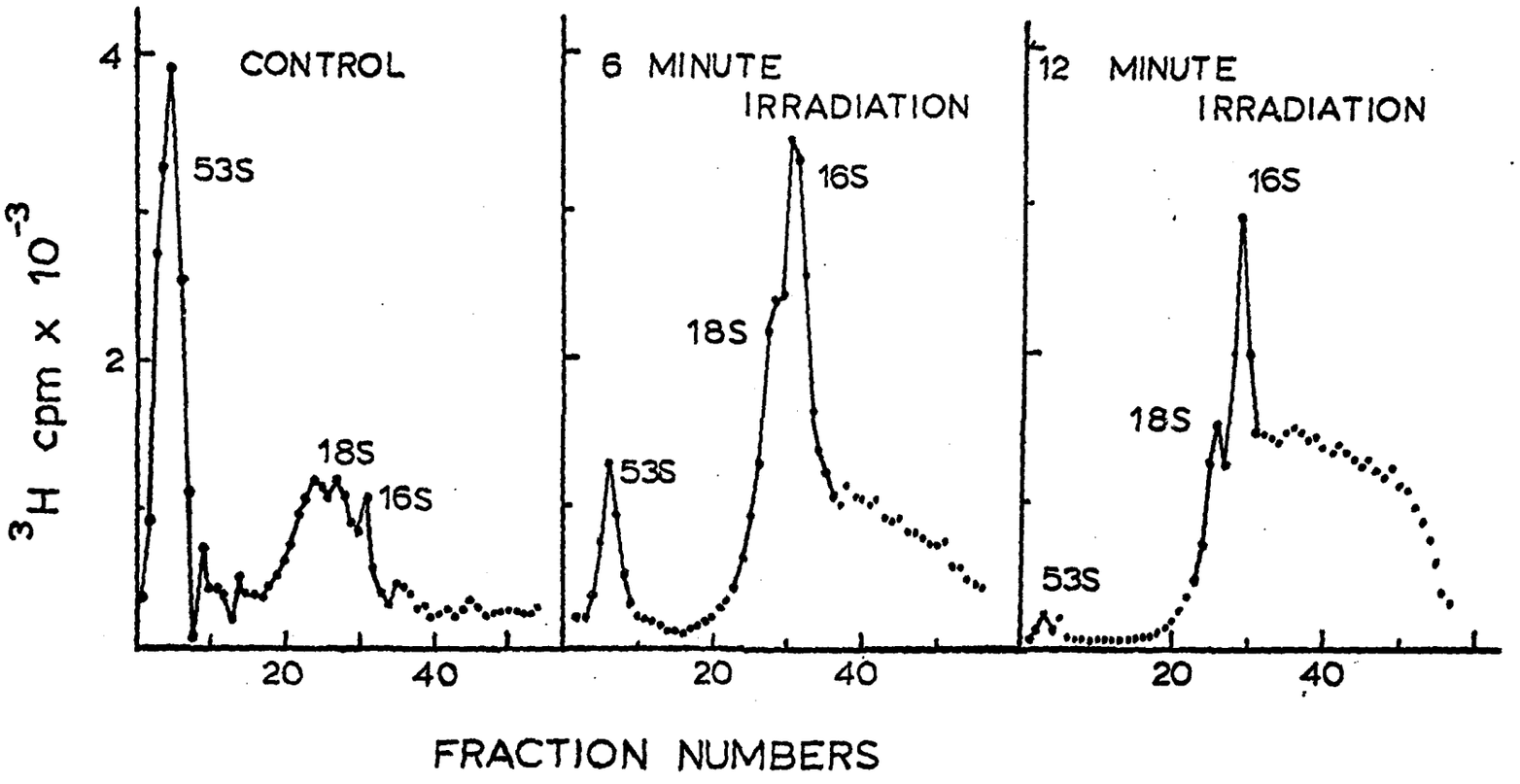
FRACTION NUMBER


CONTROL
6 MINUTE IRRADIATION
12 MINUTE IRRADIATION
53S
18S
16S
^3H cpm x 10^{-3}
4
2
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